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Pathogenesis and genetic basis for retinopathy of prematurity

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1. ABSTRACT

Retinopathy of prematurity (ROP) is a vasoproliferative disorder affecting preterm infants with low gestational age and birth weight. In general more than 50 % of preterm infants weighing less than 1250 g at birth show evidence of ROP and about 10 % of the infants develop stage 3 ROP. However, retinal detachment occurs and leads to visual loss in only a few percent of infants with stage 3 or more severe ROP, and in most cases, spontaneously regresses. The most conspicuous question is why ROP in some premature infants progresses despite rigorous and timely intervention while in other cases with similar clinical characteristics it regresses. Genetic differences between the infants could be an explanation. Although many causative factors, like low birth weight, low gestational age and supplemental oxygen therapy are associated with ROP, several indirect lines of evidence suggest the role of a genetic component in the pathogenesis of ROP. The incidence of ROP is more frequent in white than in black infants and in males than in females. Genetic polymorphism may alter the function of the genes which normally control retinal vascularization, such as vascular endothelial growth factor (VEGF), which may also be involved in pathogenesis of ROP. Evaluation of candidate genetic polymorphism influencing the outcome of ROP may provide new information about the pathogenesis of the disease. Screening of genetic polymorphisms may also help to identify and treat the high risk infants in time.

2. INTRODUCTION

Retinopathy of prematurity (ROP) is a leading cause of impaired vision and blindness in children throughout the world. It is a retinal vascular disease that occurs in infants with low gestational age and birth weight (1, 2). ROP is characterized by retinal neovascularisation, which possibly leads to retinal detachment, macular folds, myopia, refractive amblyopia, or strabismic amblyopia and may result in visual loss or blindness (3-8).

ROP is becoming more frequent due to the improved survival of extremely premature infants (9). Several etiologic factors including exposure to oxygen have been implicated in the pathogenesis of ROP. Although the exact mechanism of ROP is not yet fully understood, indirect lines of evidence suggest the possible role of a genetic component. Genetic factors such as polymorphisms of different genes may alter not only the risk but also the progression of ROP (11-15).

Monitoring of the genetic factors may help to identify infants with a high risk of advanced ROP. The CRYO-ROP randomized controlled trial demonstrated that early detection followed by cryotherapy or laser photocoagulation treatment may decrease the incidence of adverse structural outcome and visual loss by approximately 50 % (2).

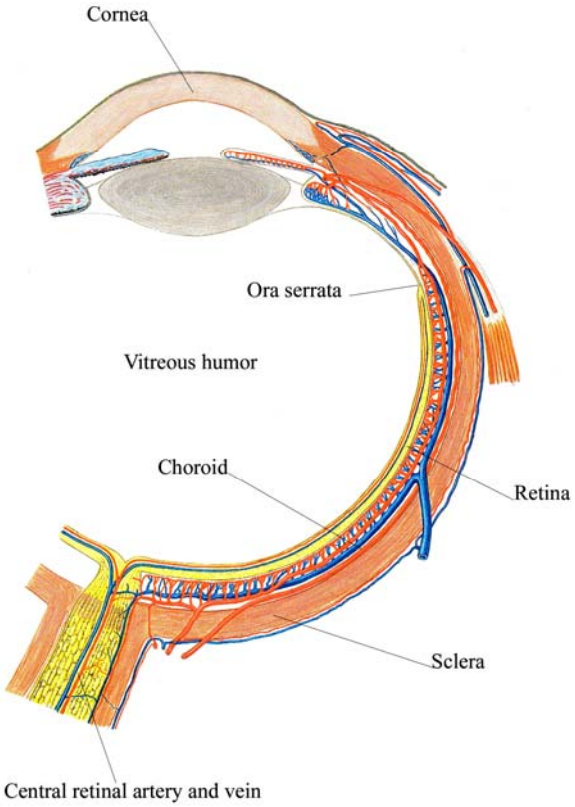

Figure 1. Schematic diagram of blood supply of the eye. The figure shows the blood vessels of the eye. Reproduced with permission with modification from: 17.

In this review we discuss the definition of ROP, the grades (severity) of ROP, the pathophysiology and risk factors associated with ROP. We also discuss the significance of the genetic background of ROP with particular focus on the Norrie disease (ND) and VEGF genes.

3. PATHOGENESIS

ROP, previously called retrolental fibroplasia, is a developmental abnormality of the retina that occurs in premature and low birth weight infants. Two distinct vascular systems supply the eye with oxygen and other nutritients. Retinal vessels supply the inner part of the retina and the choroid supplies the outer layer including photo-receptors (Figure 1 and 4A) (16, 17). Development of the choroid is completed during the third month of gestation (18), but the retinal vessels are still undeveloped in preterm infants (19). Normally, retinal vascular development begins in the 16th week of gestation. The developing blood vessels grow centrifugally from the optic disc and reach the nasal ora serrata in the 32nd week of gestation and the temporal ora serrata 8 weeks later (20).

While the retinal blood flow (RBF) is relatively low (21) the choroid blood flow (CBF) is high, with low oxygen extraction, which makes the choroid important in the oxygenisation and nutrition of the retina (21-23). In adults, RBF and CBF are maintained constant over a wide range of perfusion pressure (24-29). However, autoregulation of RBF (21) and CBF (21, 30, 31) is almost absent in preterm newborns. Consequently, when blood pressure is increased, as it often happens due to different iatrogenic manipulations (32), RBF and especially CBF, as well as oxygen delivery, increases.

Autoregulation of retinal blood flow also responds to changes in the oxygen tension of blood both in adults and newborns (31, 33-37). However, in preterm infants the choroidal circulation fails to autoregulate in response to altered oxygen tension. Under hyperoxic conditions, the choroidal vessels cannot constrict, therefore oxygen moves from the choroidal to the retinal circulation (38).

The course of ROP can be separated into two phases. The first acute phase of ROP is due to the impaired autoregulation of the retinal blood vessels and the sudden postnatal increase of oxygen tension. Neonates have an incompletely vascularized retina with a peripheral avascular zone. The relative hypoxia of the avascular zone induces normal vessel growth in part by increased synthesis of VEGF. After premature birth, as a result of the postnatal increase of oxygen tension, the normal *in utero* VEGF driven development of retinal vasculature ceases (9). The excessive delivery of oxygen leads to the generation of damaging free radicals (reactive oxygen species - ROS). Free radicals and the restricted ability of the newborns to inactivate ROS may lead to the constriction and finally to the obliteration of some of the developed vessels (10).

The second phase is hypoxia driven and depends on the response of the retina, and is characterized by normal vascularization or abnormal neovascularization (9). Decreased perfusion of the ocular vessels occurs with retinal hypoxia. In the second phase of ROP the relatively low tissue oxygenation leads to upregulation of the synthesis of different growth factors, such as VEGF and insulin-like growth factor 1(IGF-1). Finally, this upregulation of the growth factors may lead to inflammation, abnormal angiogenesis, fibrosis and retinal detachment (39-43).

In most cases ROP can spontaneously regress, however, it may often result in visual impairment or blindness (44, 45).

4. CLASSIFICATION OF ROP

International classification of ROP (ICROP) was made by an International Committee (46, 47). ROP is categorized by three features: stages for description of the severity, zones for the location and clock hours of retina for extent of ROP (Figure 2). ROP has been divided into five stages (Table 1) and is categorized by the highest stage and the lowest zone for each eye (16).

For the location of ROP the retina is divided into three zones, which are centered on the optic disc (Figure 2) (16, 44). Zone I is the most central zone. Its center is the

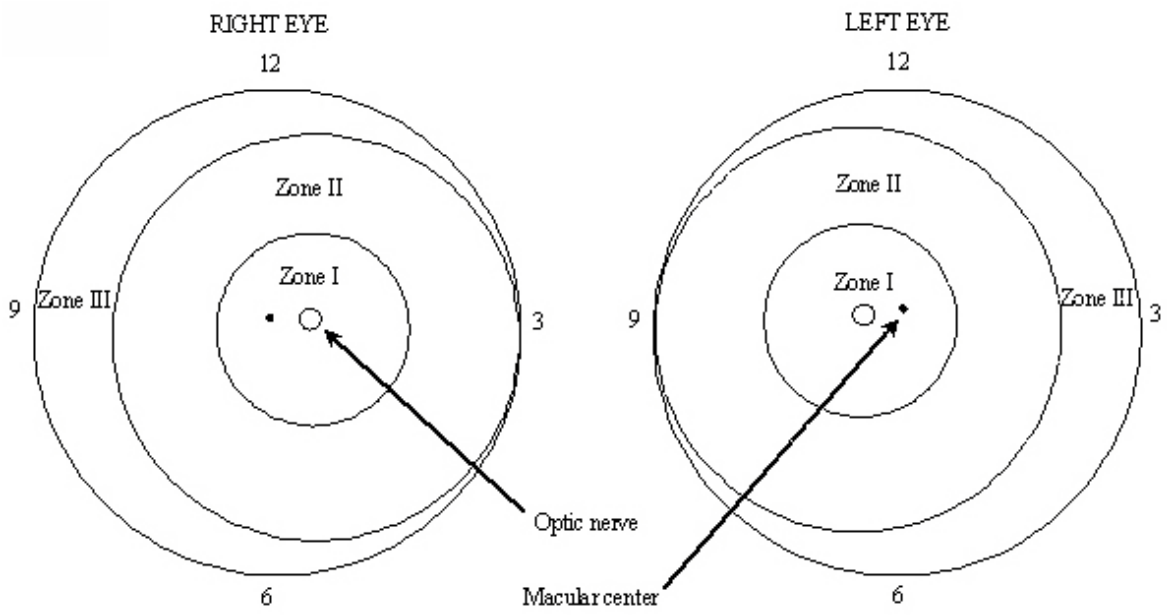

Figure 2. The posterior (Zone I), the intermediate (Zone II) and the peripheral (Zone III) zone of the retina.Grading of retinopathy of prematurity according to the three zones centered on the optic disc.

Table 1. Definition of the different stages of ROP

Stage 1	There is only a fine demarcation between the vascularized and nonvascularized areas. This line of demarcation does not have thickness or height. Abnormal branching of the blood vessels may be seen
Stage 2	The demarcation line develops into a wide and thick ridge that separates the vascular and the avascular areas of the retina (Figure 4B)
Stage 3	Neovascularization (fibrovascular proliferation) is present on the posterior surface of the ridge or ahead toward the vitreous cavity. Retinal or vitreous hemorrhage may also develop
Stage 4	Subtotal retinal detachment is present at the ridge. The retina is pulled into the vitreous by the fibrovascular ridge
Stage 5	Total retinal detachment is present

ROP is divided into stages (1 to 5) which describe the severity of the diseases. The stages are of increasing severity.

optic nerve and its radius is twice the distance from the optic nerve to the macula. ROP in zone I is always critical and must be closely monitored because changes can occur quickly within a few days. Zone II is the intermediate zone, surrounding zone I. Its nasal border is the nasal ora serrata. ROP in this area also may progress quickly; however, usually there are warning signals, such as increased vascular arcading or vascular dilation. Zone III is the most peripheral zone. It is a temporal crescent that the circle of zone II did not encompass. Serious disease is rare in this zone.

The terms “plus” and “threshold” are descriptive terms often used to further delineate the severity of disease. Plus disease can occur at any stage of ROP and is characterized by dilation or tortuosity of the posterior pole retinal blood vessels. If plus disease is present than it is likely that the condition will progress (16). Threshold disease is a term used to describe an advanced stage of ROP which requires therapeutic intervention (16).

5. TREATMENT

The treatment for ROP depends on the stage and location of the disease. Stage 1 and 2 ROP usually require only observation of the infant (16).

Laser- or cryotherapy is routinely used for the treatment of more advanced (i.e. stage 3) ROP. However, today, lasertherapy for ROP is the preferred treatment and cryotherapy is reserved for cases when blood or haze inhibits the effectiveness of laser photocoagulation (16, 44, 48). Lasertherapy reduces the oxygen demand of the tissues by destroying small parts of the retina. Moreove, it eliminates the abnormal blood vessels before they cause retinal detachment.

If a retinal detachment occurs, scleral buckling or vitrectomy may be done. Scleral buckles are usually performed in stage 4 or 5 ROP. Scleral buckling is a procedure whereby a silicone band is placed around the equator of the eye (49). This brings the retina back in contact with the inner layers of the eye. Vitrectomy is performed only in infants with stage 5 ROP (49). During vitrectomy, small incisions are made on the sclera and the vitreous is replaced with saline solution to maintain the shape of the eyeball. Scar tissue also should be cut to allow the retina to relax.

6. GENETIC BACKGROUND

ROP is a multifactorial disorder characterized by retinal neovascularization, which may lead to retinal

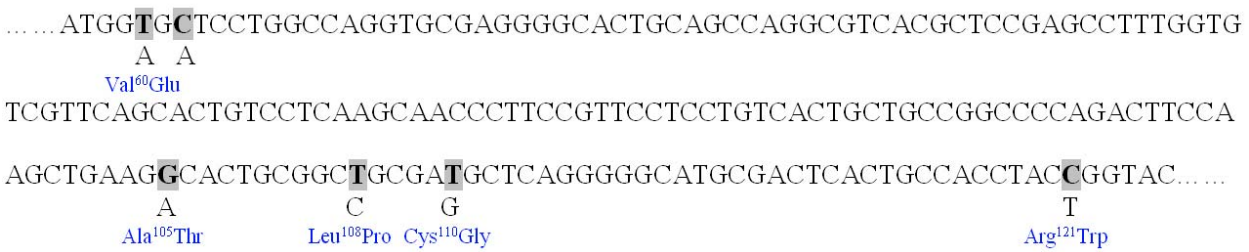

Figure 3. The partial sequence of the third exon of the Norrie disease gene. The figure shows the partial nucleotid sequence of the third exon of the Norrie disease gene and the investigated singele nucleotid polimorphisms (bold capital letters, highlighted by gray colour). This partial sequence starts with the 175th nucleotide from the first adenine nucleotide of the start codon. The changes in the nucleotid (capital letters) and in the amino acid (blue letters) sequences are given below the partial sequence of the ND gene. In the case of $C^{+597}A$ polimorphism there is no change in the sequence of amino acids.

detachment. ROP is strongly correlated with low birth weight and gestational age (1, 2), however, other factors such as high levels of supplemental oxygen (1), mechanical ventilation (50), anemia (51), intraventricular hemorrhage (52), sepsis (52, 53), concurrent illnesses (50) or maternal preeclampsia (50), have also been implicated in the pathogenesis of ROP.

Although thus far, no genetic abnormalities have been identified in the pathogenesis of ROP, several indirect lines of evidence suggest the possible role of a genetic component. The incidence of ROP varies between different races (2, 54, 55). ROP is more frequent in white than in black infants in both the USA (2) and the UK (54) and the incidence of ROP is also higher in males than in females (56). Moreover, the frequency of ROP also varies between the different countries (50, 57-63). All these facts may underlie the importance of genetic factors in the pathogenesis of ROP. The most conspicuous question is, however, why ROP in some premature infants progresses despite rigorous and timely intervention while in other cases, with similar clinical characteristics, it regresses spontaneously. Recently, several studies have been undertaken on the hypothesis that genetic polymorphism may alter the course of ROP.

It is likely that factors that normally control retinal vascularisation may also be involved in the pathogenesis of ROP. Regarding the genetic polymorphism in the pathogenesis of ROP the two most frequently investigated genes are the Norrie disease (ND) and VEGF.

6.1. Polymorphism of Norrie disease gene

Developmental retinal diseases such as ROP, ND and X-linked familial exudative vitreoretinopathy (FEVR) have several similar clinical manifestations. All of them exhibit retinal detachment, retinal traction and fibrovascular membrane formation (9, 64, 65). ND and X-linked FEVR have been shown to involve mutations of the ND gene (66-68). The ND gene (GenBank, gene accession numbers: NM_000266) consists of three exons, which encode a small secreted protein, which is expressed mainly in the brain and retina (69, 70). Norrin the product of the ND gene plays a role in the angiogenesis (71) of the developing retina (72, 73). Indeed, Norrie disease gene mutant mice that are deficient in norrin develop blindness (74), show a distinct failure in retinal angiogenesis, and completely lack the deep capillary layers of the retina (76).

Sharsty *et al* identified two missense mutations ($Leu^{108}Pro$, $Arg^{121}Trp$) in the third exon of the ND gene (Figure 3) in white premature infants with birth weights less then 1,500 g in the USA. Although, Sharsty *et al* investigated only a limited number of premature infants these were the first results suggesting that genetic factors may play a role in the development of ROP. They suggested that these mutations may play a role in the development of threshold ROP (76). However, later Haider *et al* was no able to demonstrate this association in a larger population of Kuwaiti Arab preterm infants with ROP (77).

Beside these missense mutations ($Leu^{108}Pro$, $Arg^{121}Trp$) Haider *et al* also investigated four different polymorphisms ($C^{+597}A$, $Val^{60}Glu$, $Ala^{105}Thr$, $Cys^{110}Gly$) of the third exon of the ND gene in Kuwaiti Arab infants (Figure 3) (13, 14). The incidence of the different genotypes of the ND gene in the control and ROP cases are summarized in Table 2. Two hundred and ten premature newborns were investigated, 115 newborns had no eye problems (controls) and 95 had different stages of ROP. The mean gestational age of the infants was 30.7 weeks (range 27-37) and the mean birth weight was 1267 g (range 800-1500 g).

The ND $^{+597}$CC genotype was present in 64 % of the ROP and also in the control cases. Meanwhile, in the cases of premature newborns who were treated because of threshold ROP only 4 % had $^{+597}$CC genotype and the majority (83%) had $^{+597}$AA genotype. In whom ROP spontaneously regressed, 85 % had $^{+597}$CC genotype while no one had the $^{+597}$AA genotype. The differences in the distribution of the three genotypes ($^{+597}$CC, $^{+597}$CA, $^{+597}$AA) between ROP and control cases and also between threshold and spontaneously regressed ROP cases were statistically significant (χ^2, both $p<0.0001$) (Table 2).

There was, however, no significant difference in the distribution of the other three genotypes of the ND gene ($Val^{60}Glu$, $Ala^{105}Thr$ and $Cys^{110}Gly$) between cases of ROP and controls and also between the threshold and spontaneously regressed ROP cases (Table 2).

Table 2. Genotype distribution for the studied polymorphism of Norrie disease (ND) gene

	Controls	**ROP cases**		
		Total	Spontaneous regression	Threshold ROP
ND $C^{+597}A$				
CC	74 (64)	61 (64)	60 (85)	1 (4)
CA	29 (25)	14 (15)	11 (15)	3 (13)
AA	12 (11)	20 (21)	0 (0)	20 (83)
ND $Val^{60}Glu$				
Val Val	109 (95)	84 (89)	65 (92)	19 (79)
Val Glu	5 (4)	6 (6)	4 (5)	2 (8)
Glu Glu	1 (1)	5 (5)	2 (3)	3 (13)
ND $Ala^{105}Thr$				
Ala Ala	110 (95)	83 (87)	63 (89)	20 (83)
Ala Thr	3 (3)	10 (11)	7 (10)	3 (13)
Thr Thr	2 (2)	2 (2)	1 (1)	1 (4)
ND $Cys^{110}Gly$				
Cys Cys	107 (93)	93 (98)	69 (97)	24 (100)
Cys Gly	2 (2)	0 (0)	0 (0)	0 (0)
Gly Gly	6 (5)	2 (2)	2 (3)	0 (0)
Study	Haider M.Z. *et al.* (12, 13)			

Data shows the percentages of the different Norrie disease (ND) genotypes given in parentheses and the number of the investigated patients. Genetic polymorphisms were compared between ROP (total) and control (controls) cases and also between preterm infants with threshold ROP (Threshold ROP) and spontaneously regressed cases (spontaneous regression). In case of $C^{+597}A$ and $Cys^{110}Gly$ the mean birth weight of the preterm infants was 1267 g (range: 800-1500 g) and the mean gestational age was 30.7 weeks (range: 26-36 w). In case of $Val^{60}Glu$ and $Ala^{105}Thr$ polymorphisms the gestational age was 32 weeks or less and the birth weight was below 1500g. The differences in the distribution of the three genotypes of ND $C^{+597}A$ between ROP and control cases (χ^2, p=0.0001) and also between threshold and spontaneously regressed ROP cases (χ^2, p=0.0001) were statistically significant.

Haider *et al* concluded that while ND $C^{+597}A$ polymorphism may play a role in the progression of ROP, $Val^{60}Glu$, $Ala^{105}Thr$, $Cys^{110}Gly$ polymorphisms are not associated with ROP or the risk of progression of the disease to advanced stages.

6.2. Polymorphism of vascular endothelial growth factor (VEGF) gene

The formation of the primary vascular network in the retina happens due to the influence of tissue oxygenation and VEGF released by astrocytes (78, 79). VEGF is a hypoxia induced potent angiogenic factor (80, 81) which induces proliferation (82) and migration (83) of endothelial cells. The importance of VEGF in the development of ROP is supported by a number of experimental and clinical data. Indeed, decreased VEGF production was demonstrated during the first phase (84) while it increased during the proliferative, second phase of ROP (40, 85). Increased VEGF synthesis and high levels were observed in hypoxic retina (86, 87) and in other experiments the inhibition of VEGF prevented retinal ischemia-associated neovascularization (88, 89).

The VEGF gene is highly polymorphic (GenBank, gene accession numbers: AF095785, AF437895), however, most of the polymorphisms are rare or not functional. Recently, two independent studies investigated the role of the functional polymorphism of VEGF in DNA samples of preterm infants born before the 32nd week and with birth weights below 1500 g (11, 12) (Table 3). Newborns who were treated because of threshold ROP were compared to newborns with no or only mild ROP (controls).

The incidences of the different genotypes of the VEGF gene in the control and threshold ROP cases are summarized in Table 3. Cooke *et al* found significantly different genotype distribution of VEGF $G^{-634}C$ polymorphism in threshold and control ROP cases (Yates corrected χ^2, p=0.03) and significantly higher proportion of the VEGF ^{-634}C allele in the control ROP cases (odds ratio (95% CI): 2.0 (1.11-3.69), p<0.05) (11). Interestingly in an other study Vannay *et al* demonstrated higher prevalence of VEGF ^{-634}C allele in threshold than in control ROP cases (p<0.05), and that the heterozygous and homozygous carrier state of VEGF ^{+405}C alleles presented an independent risk factor for ROP (adjusted odds ratio (95% CI): 2.00 (1.02-3.92), p = 0.045 and 3.37 (1.17-9.65, p=0.011, respectively) (Table 3) (12).

There was, however, no significant difference in the distribution of $T^{-1507}C$ or $C^{+936}T$ polymorphisms of the VEGF gene between cases of threshold ROP and controls (Table 3).

These data though contradictory, suggest that the progression of ROP may be influenced by functional polymorphism of the VEGF gene (11, 12) (Table 3). They concluded that caution should be taken in the interpretation of the results and larger studies, measuring also the protein levels of VEGF, would be preferred.

Table 3. Genotype distribution for the studied polymorphisms of vascular endothelial growth factor (VEGF) gene

	Controls	**Threshold ROP**	**Controls**	**Threshold ROP**
VEGF $T^{-1507}C$ (previously VEGF $T^{-460}C$)				
Function: *In vitro* promoters carrying VEGF $^{-1507}$C allele have 71% greater activity compared with those that do not have this allele (111).				
TT	-	-	36 (31)	21 (24)
TC	-	-	63 (55)	45 (53)
CC	-	-	16 (14)	20 (23)
VEGF $G^{-634}C$ (previously VEGF $G^{+405}C$)				
Function: High serum levels of VEGF in patients homozygous for VEGF $^{-634}$C alleles (110). High VEGF levels in LPS induced leukocytes with $^{-6345}$GG genotype (113).				
GG	30 (31)	43 (48)	41 (48)	40 (35)
GC	52 (55)	38 (43)	40 (46)	55 (48)
CC	13 (14)	8 (9)	5 (6)	20 (17)
VEGF $C^{+936}T$				
Function: VEGF $^{+936}$T have been shown to decrease VEGF production *in vitro* and *in vivo*, respectively (114).				
CC	65 (70)	64 (74)	-	-
CT	20 (21)	18 (21)	-	-
TT	8 (9)	4 (5)	-	-
Study	Cooke R.W. *et al* (11)		Vannay A. *et al* (12)	

Data shows the percentages of the different vascular endothelial growth factor (VEGF) genotypes given in parentheses and the number of the investigated patients. Genetic polymorphisms was compared between preterm infants treated (threshold ROP) and not treated (controls) due to advanced ROP. All infants were born before the 32nd week of gestation and with a birth weight below 1500 g. Cooke *et al* found different genotype distribution of VEGF $G^{-634}C$ polymorphism in threshold and control ROP cases (Yates corrected χ^2, p=0.03) and significantly higher proportion of the VEGF $^{-634}$C allele in the control ROP cases (Odds ratio (95% CI): 2.0 (1.11-3.69)). Vannay *et al* demonstrated higher prevalence of VEGF $^{-634}$C allele in threshold tnan in control ROP cases ($p<0.05$) and that the heterozygous and homozygous carrier state of VEGF $^{+405}$C alleles presented an independent risk factor for ROP (adjusted odds ratios (95% CI): 2.00 (1.02-3.92), p = 0.045 and 3.37 (1.17-9.65, p=0.011, respectively).

6.3. Polymorphism of angiotensin converting enzyme (ACE), transforming growth factor (TGF) beta-1 and tumor necrosis factor (TNF) alphạgene

Beside genetic polymorphism of ND and VEGF some other genes such as angiotensin converting enzyme (ACE), transforming growth factor (TGF) beta-1 and tumor necrosis factor (TNF) alpha were also investigated.

ACE is a component of the renin-angiotensin system that hydrolyses angiotensin I to generate angiotensin (ANG) II. ANG II is a key pathophysiological factor in a number of retinal vascular disorders including ROP and proliferative diabetic retinopathy (90, 91). In addition to its well characterized hemodynamic actions, ANG II is also a potent endothelial and smooth muscle cell proliferation (92, 93) and smooth muscle cell hypertrophy factor (94).

Haider *et al* investigated a functional insertion/deletion (I/D) polymorphism of the ACE gene in preterm Kuwaiti infants (Table 4) (15). This polymorphism consists of the presence or absence of a 250bp long DNA fragment. The serum ACE concentration is the highest in homozygotes with the shorter allele (DD) compared to heterozygotes (ID) or to homozygotes with the longer allele (II) (15, 95).

One hundred and eighty one newborn infants born before the 32nd week with birth weights below 1500 g were investigated. One hundred and seven had no eye problems (birth weight: 1210 g (range: 710-1500 g); gestational age: 30.5 weeks (range: 24-32 w)) (controls) and 74 had different stages of ROP (birth weight: 1180 g (range: 700-1500 g); gestational age: 30.2 weeks (range: 25-32 w)).

The incidence of DD genotype was similar in the total ROP and control, non-ROP cases. They found lower incidence of the ID genotype and significantly higher incidence of II genotype in the ROP cases compared to non-ROP controls (24 % vs. 7 %, Yates corrected χ^2, p=0.01). Interestingly, when threshold ROP cases were compared to spontaneously regressed ROP cases the incidence of DD genotype was significantly higher in threshold ROP cases (62 % vs. 36 %, Yates corrected χ^2, p=0.04) (Table 4).

Haider *et al* suggested that genotype II of the ACE gene may be a risk factor for the onset of ROP and that the presence of DD genotype in ROP patients may present a higher risk for the development of advanced stages of ROP. They suggested that in premature infants the presence of D allele, which has been associated with higher tissue levels of ACE may trigger vasoconstriction, proliferation or angiogenic factors thereby inducing neoangiogenesis.

A recent study investigated the incidence of polymorphisms of TGF beta-1 ($C^{-509}T$) and TNF alphạG^{-308}Ạ in preterm infants born before the 32nd week and with

Table 4. Genotype distribution for the studied polymorphisms of angiotensin converting enzyme (ACE) gene

	Controls	**ROP cases**		
		Total	Spontaneous regression	Threshold ROP
ACE I/D				
Function: The serum ACE concentration is the highest in homozygotes with the shorter allele (DD) compared to heterozygotes (ID) or to homozygotes with the longer allele (II) (15, 94); D allele is associated with higher risk of vascular disorder				
DD	45 (42)	32 (43)	19 (36)	13 (62)
ID	54 (51)	24 (33)	19 (36)	5 (24)
II	8 (7)	18 (24)	15 (28)	3 (14)
Study	Haider M.Z. *et al.* (15)			

Data shows the percentages of the different angiotensin converting enzyme (ACE) gene genotypes given in parentheses and the number of the investigated patients. Genetic polymorphisms were compared between ROP (total) and control (controls) cases and also between preterm infants with threshold ROP (Threshold ROP) and spontaneously regressed cases (spontaneous regression). All infants were born before the 32nd week of gestation and with a birth weight below 1,500 g. Incidence of II genotype higher in the ROP cases compared to non-ROP controls (24 % vs. 7 %, Yates corrected χ^2, p=0.01). Incidence of DD genotype was significantly higher in threshold ROP compared to spontaneously regressed cases (62 % vs. 36 %, Yates corrected χ^2, p=0.04)

Table 5. Genotype distribution for the studied polymorfism of transforming growth factor (TGF) beta-1 and tumor necrosis factor (TNF alpha gene

	Controls	**Threshold ROP**
TGF beta-1 $C^{-509}T$		
Function: has been shown to correlate with *in vitro* protein level		
CC	47 (54)	41 (49)
CT	33 (38)	35 (42)
TT	7 (8)	8 (9)
TNF alpha $G^{-308}A$		
Function: TNF alpha $G^{-308}A$ has been shown to influence the supplemental oxygen requirement (104) and associated with increased sepsis mortality in very low birth weight infants (105).		
GG	59 (63)	55 (64)
GC	32 (34)	27 (31)
CC	3 (3)	4 (5)
Study	Cooke R.W. *et al* (11)	

Data shows the percentages of the different transforming growth factor (TGF) beta-1 and tumor necrosis factor (TNF) alpha genotypes given in parentheses and the number of the investigated patients. Genetic polymorphisms were compared between preterm infants treated (threshold ROP) and not treated (controls) due to advanced ROP. All infants were and born before the 32nd week of gestation and with a birth weight below 1500 g.

birth weights below 1500 g (11). Newborns who were treated because of threshold ROP were compared to newborns with no or only mild ROP (controls).

TGF beta-1 and TNF alpha are multifunctional cytokines involved in the proliferation (96), differentiation (97), migration (98, 99), and survival of different cell types (100, 101). TGF beta-1 and TNF alpha also play a role in the regulation of angiogenesis (102, 103). Serum TNF alpha levels also have been shown to correlate with the progression of diabetic retinopathy (104).

The investigated TGF beta-1 $C^{-509}T$ polymorphism has been shown to correlate with the TGF beta-1 protein level (105). *In vitro* experiments also have demonstrated that TNF alpha $G^{-308}A$ polymorphism has direct effects on TNF alpha gene regulation (106). Moreover, TNF alpha $G^{-308}A$ has been shown to be associated with increased sepsis mortality in very low birth weight infants (107).

However, on the basis of the study by Cooke *et al* it appears that these polymorphisms do not appear to be a risk factor for developing advanced stage ROP (Table 5).

6.4. Summary

In summary, testing of polymorphisms, rather, the combination of different polymorphisms, would provide valuable information for the risk assessment of ROP. However, caution should be taken in the interpretation of studies of genetic polymorphisms. It is important that the investigated population be well defined, considering the different races. Large studies including the measurment of protein levels in association with the genotyping analyses would be the preferred approach.

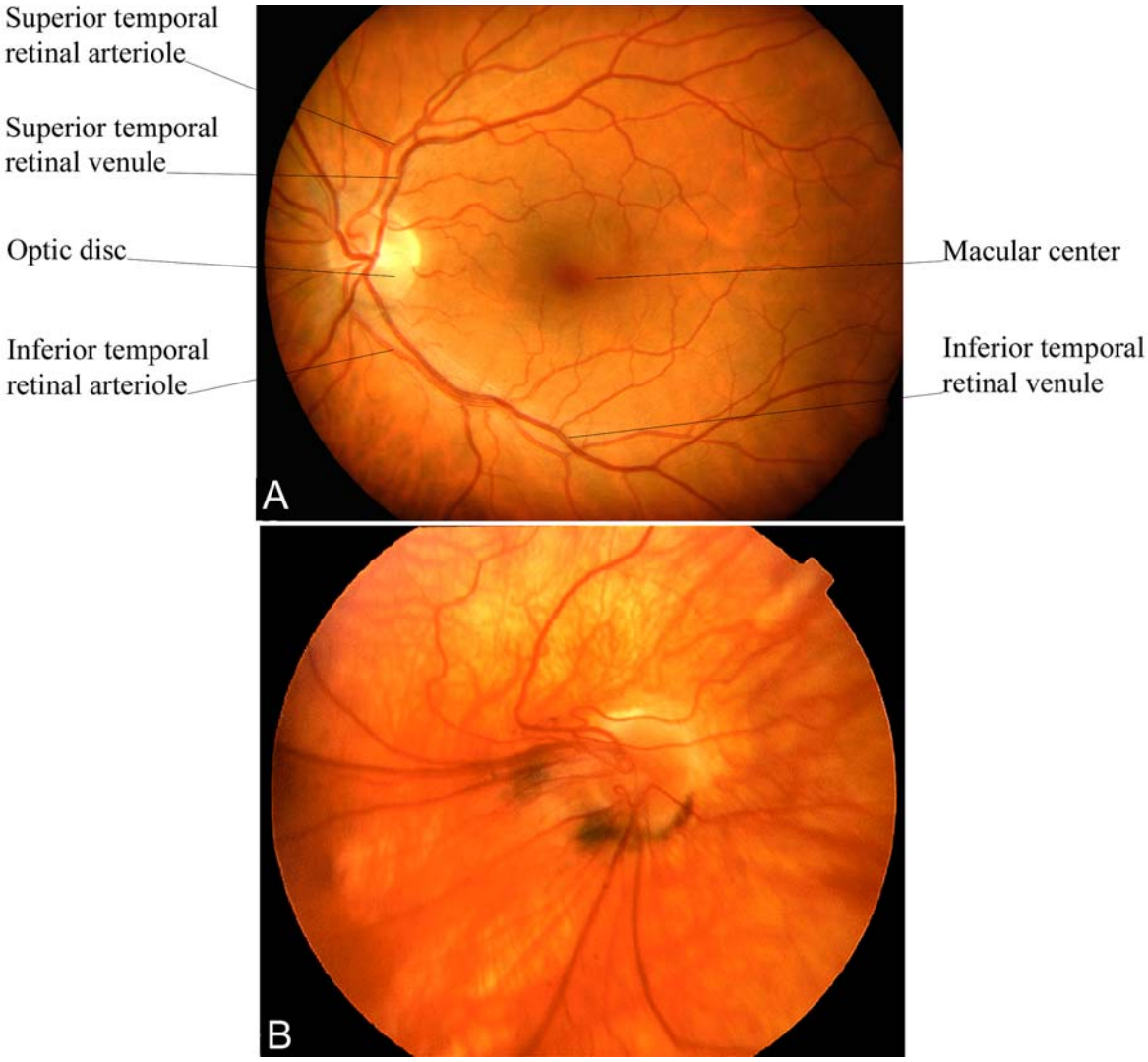

Figure 3. A normal fundus and those of premature infants with ROP. A. Shows the normal vascular structure of a fundus (Provided by Dr Éva Széles, Department of Ophthalmology, Semmelweis University, Budapest, Hungary); B. Dragging of disc vessels in retinopathy of prematurity stage 2; (Provided by Dr. Chung Nen Chua, BMedSci MRCP FRCOphth, UNIMAS and Kuching General Hospital, Kuching, Sarawak, East Malaysia).

7. PERSPECTIVES

Recent studies suggest the importance of early identification of the population at risk of developing advanced ROP. It has been demonstrated that early treatment may decrease the incidence of permanent visual loss of the infants (2).

The evolution of molecular genetics and molecular biology has raised the standard of ROP research. Different genetic polymorphisms, which may be risk factors for advanced ROP have been identified. Monitoring of these genetic factors may help in the identification and timely treatment of infants at high risk.

Several new strategies have recently been developed to prevent and treat ROP. In a previous study, intraocular administration of recombinant human monoclonal antibody against VEGF was able to prevent the choroidal neovascularization in monkeys (108). In another study, neutralization of VEGF receptor-2 with a blocking antibody inhibited the pathological neovascularization, but had no effect on normal angiogenesis in a canine model of ROP (109). The use of anti VEGF aptamers (110) may also open up exciting possibilities for the treatment of ROP.

Today we understand much more about the nature of ROP that may help to change our approach to this disease. An ideal therapy would be one that could be administered with minimal invasiveness that would prevent neovascularization, and would be without any other adverse effect on the normal development of blood vessels. Perhaps in the next few years we will pay more attention to prevention and targeted treatment of ROP.

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